Red Cell Disorders

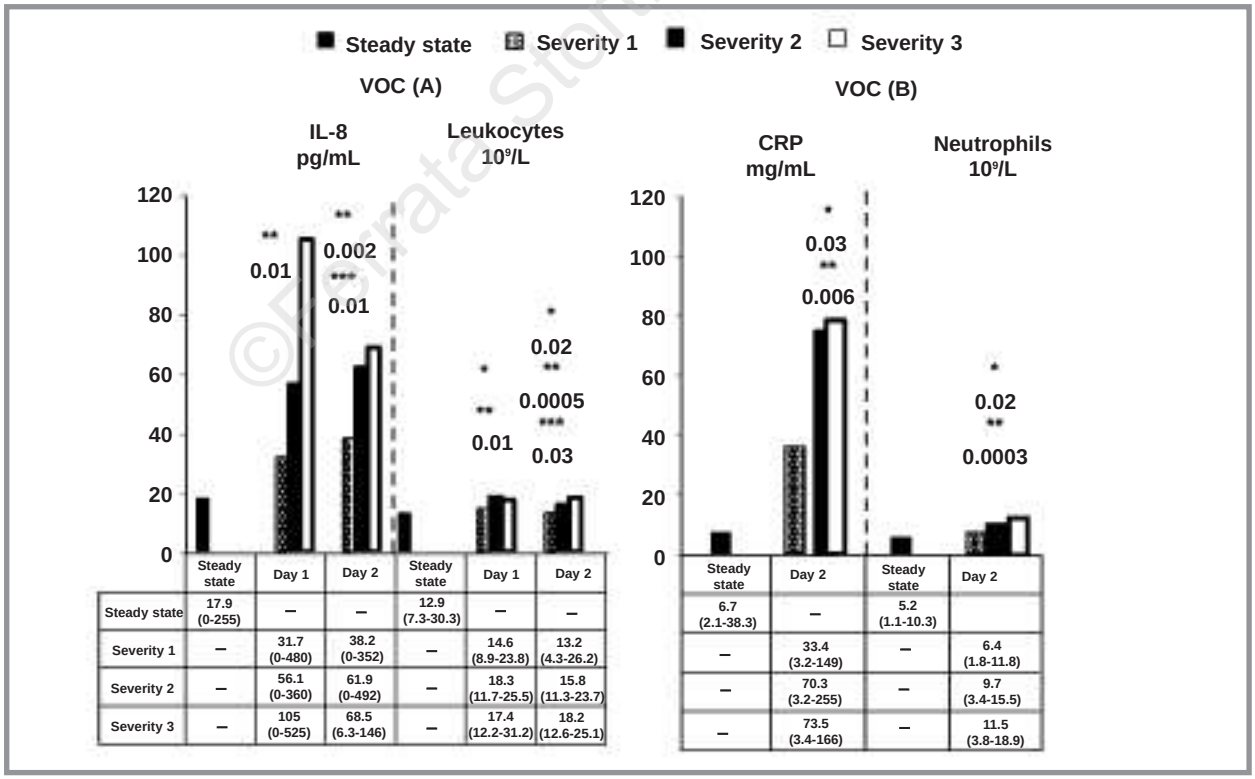
Childhood sickle cell crises: clinical severity, inflammatory markers and the role of interleukin-8

There is emerging consensus that a pro-inflammatory condition contributes to the vaso-occlusive complications of sickle cell disease (SCD).[1-4] We evaluated the potential value of inflammatory mediators as early markers of severity of painful vaso-occlusive crises (VOC) in SCD. We assayed the plasma levels of cytokines, soluble vascular cell adhesion molecule-1, acute phase proteins, secretory phospholipase and standard hematologic indices.

haematologica 2004; 89:863-864
(*http://www.haematologica.org/2004/7/863*)

We analyzed 58 VOC that developed in 34 children with sickle cell anemia (Hb SS); 23 of these children were also studied during steady state. The median age of the patients was 8.6 years (range: 1 to 18 years). Clinical data and samples were systematically collected at admission (day 1), 24 hours later (day 2) and in the steady state, i.e. at least two months away from any acute episode. A control group of 55 healthy normal children (Hb AA), matched for age, sex and race with the SCD group, was also studied. Full written consent was obtained prior to the subjects' recruitment in the study, which was approved by the local ethical committee. Biological evaluations included (i) hematologic parameters (STKS electronic cell counter); (ii) inflammatory markers: C-reactive protein (CRP) (nephelometric assay, Berhing BNII analyzer) and α1-glycoprotein (radial immunodiffusion, Berhing BN100 analyzer), (iii) soluble vascular cell adhesion molecule-1 (sVCAM-1), (iv) secretory phospholipase A2 (sPLA2), and (v) cytokines: interleukin-6 and interleukin-8 (ELISA, according to the manufacturers' instructions). For the purpose of this study an Episode Severity Index (ESI) was defined according to five objective data (Table 1). In parallel, a clinical level of severity was attributed independently by two physicians to each episode. *Severity 1* corresponded to mild episodes which could have been treated at home or in a day-care center, *severity 2* to moderate or severe episodes that were never a risk to life and *severity 3* to severe and life-threatening episodes. EpiInfo 6.04 software was used for univariate data analysis. The significance of results was evaluated by non-parametric Mann-Whitney or Kruskal-Wallis tests. Statistical significance was set at $p < 0.05$.

Severities 1, 2 and 3 were significantly related to each parameter included in the ESI (Table 1): zenith of pain quotation (p=0.0003), analgesic level ($p < 10^{-6}$), level 3 analgesia duration ($p < 10^{-6}$), episode duration ($p < 10^{-6}$) and patient's behavior during episode ($p < 10^{-6}$). Moreover, the mean value of the ESI, calculated by



	Steady state	Day 1	Day 2	Steady state	Day 1	Day 2
Steady state	17.9 (0-255)	–	–	12.9 (7.3-30.3)	–	–
Severity 1	–	31.7 (0-480)	38.2 (0-352)	–	14.6 (8.9-23.8)	13.2 (4.3-26.2)
Severity 2	–	56.1 (0-360)	61.9 (0-492)	–	18.3 (11.7-25.5)	15.8 (11.3-23.7)
Severity 3	–	105 (0-525)	68.5 (6.3-146)	–	17.4 (12.2-31.2)	18.2 (12.6-25.1)

	Steady state	Day 2	Steady state	Day 2
Steady state	6.7 (2.1-38.3)	–	5.2 (1.1-10.3)	
Severity 1	–	33.4 (3.2-149)	–	6.4 (1.8-11.8)
Severity 2	–	70.3 (3.2-255)	–	9.7 (3.4-15.5)
Severity 3	–	73.5 (3.4-166)	–	11.5 (3.8-18.9)

Figure 1. Symptomatic sickle cell patients: variation in biological parameters during steady state and vaso-occlusive crises (VOC) according to clinical severity. VOC (A): significant differences on days 1 and 2, VOC (B): Significant differences on day 2. Significant differences between: *severity groups 1 and 2, **severity groups 1 and 3, ***severity groups 2 and 3. The *p* values are indicated below each symbol. Significant differences from the steady state are represented by the columns with a bold border.

Table 1. Episode Severity Index (ESI): severity stratification according to five clinical parameters.

Zenith of pain quotation	*Analgesic type**	*Level 3 analgesic*	*Episode duration*	*Behavior duration*
< 40 = 1	Level 1 = 1	<24h =1	0 to 4 days =1	normal = 0
41 to 60 = 2	Level 2 = 2	24 to 72h = 2	4 to 7 days = 2	agitated = 1
61 to 100 = 3	Level 3 = 3	>72h = 3	>7 days = 3	very disturbed or too quiet = 2

**according to the WHO analgesic ladder, i.e.: Level 1: acetaminophen and acetylsalicylic acid or NSAID (ibuprofen); Level 2: 1 + codeine or nalbufine; Level 3: 1 + opioids (e.g. morphine) with or without patient-controlled analgesia.*

adding the parameter values (ranging from 4 to 14), was significantly different in the severity groups 1, 2 and 3, with respectively mean ESI values of, respectively, 5.0, 8.5 and 12.0 ($p < 10^{-6}$). These data allowed the validation of the clinical severity stratification and its use in the further analysis to reduce the number of comparisons made.

The biological data obtained during the VOC were studied according to the clinical severity and were compared to the steady-state values. Of the 58 VOC analyzed, 39.6% were severity 1 episodes, 34.5% severity 2 episodes and 25.9% severity 3 episodes; there were no deaths. Only significant variations related to the studied criteria are presented in Figure 1. As can be see from this figure, higher IL-8 levels and whole leukocyte counts were present early in severity 3 rather than in severity 1 or 2 episodes. Figure 1B, on the other hand, shows that neutrophils and CRP increased significantly in the severe episodes but in a delayed manner.

The levels of these biological parameters were statistically higher during severity 2 and 3 episodes than during the steady state (Figures 1A and B). IL-6, sVCAM-1 and sPLA2 levels were not significantly different between the 3 severity groups.

It is worth noting that the steady state data of the Hb SS patients, compared with those of the Hb AA controls, revealed higher leukocyte and neutrophil counts as well as higher CRP, α1-glycoprotein and sVCAM-1 levels (*data not shown*).

These results demonstrate, for the first time, that IL-8 is implicated in the severity of VOC episodes. These data could explain why enhanced serum levels have been found in acute chest syndrome[5] and enhanced[6,7] or undetectable serum levels in patients with VOC.[5,8] IL-8 may contribute to SCD pathology through this cytokine's ability to activate neutrophils, which are the first inflammatory cells to appear at the site of vessel damage, exacerbating and propagating inflammation. Moreover, IL-8 might also increase the adherence of sickle red cells to endothelium by activating the α4β1 integrin receptors on sickle reticulocytes.[9] The novel therapeutic approaches designed to inhibit the nuclear factor NF-κB,[10] which promotes the expression of a number of genes including those for IL-8 and IL-6, deserve further development.

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Funding: This work was supported in part by the CHU Pointe-à-Pitre, and by the Institut National de la Santé et de la Recherche Médicale (Inserm UMR S 458).

Keywords: sickle cell disease, crisis, inflammation, interleukin, severity marker.

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